PLACENTATION IN THE COW

by

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B. A., Park College, 1953

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1957

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INTRODUCTION AND REVIEW OF LITERATURE

The processes of placentation involve the development of an area of activity, the placenta, between the parent and the developing embryo. The normal mammalian placenta has been defined as "an opposition or fusion of the fetal membranes to the uterine mucosa for physiological exchange" (Mossman, 1937).

Before the phenomena involved in the processes of placentation in the cow could be comprehended, the histology of the endometrium during its cyclic changes had to be understood. The estrous cycle of the cow is well known but histological correlations are incomplete. The complete cycle has a mode length of 20 or 21 days varying with the age of the animal (Asdell, et al., 1949). Heifers tend to have a slightly shorter period than polyparous cows (Trimberger, 1948). Variations due to breed were also noted but proved to lack statistical significance.

Ovulation occurs after the end of estrus. The length of time after estrus to ovulation has been reported variously as 13.57 ± 0.6 hours (Brewster and Cole, 1941), approximately 14 hours (Malhandrov and Casida, 1942), and 10.5 hours (Trimberger, 1948).

Excellent reviews of the literature concerning the uterus of the cow have been provided by Weeth and Herman (1952) and Björkman (1954).

Histologically the endometrium has been divided (Kingman,

1944) into (1) the uterine epithelium, (2) the stratum compactum, (3) the stratum spongiosum, and (4) the stratum basalis. The uterine epithelium lines the lumen and the stratum basalis is next to the myometrium. Histological studies of the endometrium have been conducted in relation to the estrous cycle (Cole, 1930; Asdell, et al., 1949), endocrine disorders (Cupps, et al., 1956) and abnormalities (Moss, et al., 1956a). These studies have shown something of the cyclic changes in the structure of the endometrium.

Although the uterus has also been studied histologically during pregnancy, the time and method of attachment of the embryo to the uterine wall has not been adequately described and there has been no agreement between authors. Early workers (vide Amoroso, 1952; Weeth and Herman, 1952; Björkman, 1954) gave the time of attachment as between the first and second months of gestation. Winters, et al.(1942) stated that "attachment of the embryo to the uterine wall takes place during the eleventh to twelfth day." This conclusion was based on their inability to flush the embryo from the uterus at that stage of development. Other workers have given such times as 50 to 90 days gestation. (Kingman, 1948), 33 days after ovulation (Melton, et al., 1951), 40 to 50 days gestation (Chang, 1952) and 36 days of pregnancy (Foley and Reece, 1953). Björkman (1954) found that attachment was already well established in the area around the embryo by the fortieth day of gestation and

was in progress in the more distal areas.

The development of the fetal villi from the chorionic vesicle was described as starting with the formation of placental plates of serosal cells (Melton, et al., 1951). The cells of the plates formed cones which were the anlage of the cotyledons. Later the cones increased in length to form individual villi and then developed a mesodermal core as branching of the villi was started.

The fate of the uterine epithelium during pregnancy has remained unanswered. All authors have noted the presence of a layer of cells of questionable origin lining the crypts in the maternal portion of the placenta. Many of the early workers believed the cryptal lining to be of maternal origin (vide Amoroso, 1952; Weeth and Herman, 1952; Björkman, 1954). Hatch (1941) stated that the caruncular epithelium was maintained at all times and could be demonstrated as continuous. Other workers have taken the opposite view, some stating merely that the cryptal lining appears to be of fetal origin (Hammond, 1927; Amoroso, 1952; Moss, et al., 1956b) and others that the cryptal lining is definitely of fetal origin (Foley and Reece, 1953).

The classification of placentae has been attempted on various criteria. Classifications based solely upon the degree of fusion of the tissues involved were inadequate in many respects. Consequently, the classifications of Strahl and Grosser as combined and modified by Mossman (1937) have become the most widely accepted.

Terminology in regards to the bovine placenta and its related structures was found to be unsettled and changing. For example, the term "cotyledon" has been used to designate the embryonic part of the placenta, the maternal part of the placenta and even the combined placental unit.

The maternal portion of the placenta was referred to as maternal cotyledon (Mossman, 1937; Foley and Reece, 1953; Asdell, 1955), cotyledonary burr (Amoroso, 1952) but most commonly as "caruncle" (Melton, et al., 1951; Wimsatt, 1951; Weeth and Herman, 1952; Björkman, 1954). The term "caruncle" was used by these authors to designate any of the protuberences from the endometrium during pregnancy and non-pregnancy. "Caruncle" will be used throughout the following discussion to refer to any one of the small fleshy prominences or excrescences of the uterine endometrium that is or may be involved in the formation of the maternal portion of the placenta.

The embryonic portion of the placenta was referred to as fetal cotyledon (Mossman, 1937; Foley and Reece, 1951), placental plate (Melton, et al., 1951), but most usually as "cotyledon" (Wimsatt, 1951; Amoroso, 1952; Weeth and Herman, 1952; Björkman, 1954). The term "cotyledon" was used by these authors to refer to the clumps of chorionic villi on the fetal membranes. In the discussion to follow, the term "cotyledon" will be used to refer only to any one of the clusters of chorionic villi separated by smooth chorio-allantois character-

istic of the ruminants.

The placental unit of combined maternal and fetal tissue has also been referred to as the cotyledon (Mossman, 1937; Melton, et al., 1951), however, the most prevalent term has been "placentome" (Wimsatt, 1951; Amoroso, 1952; Weeth and Herman, 1952; Foley and Reece, 1953; Björkman, 1954). These authors have used the term "placentome" to indicate "any one of the unions of maternal and fetal structures (caruncles and cotyledons)" and will be used thusly during the following discussion.

"Gestation" has generally been used as synonomous with "gestation period" meaning the period from conception to parturition. The all too frequent everyday misuse of the word gestation has resulted in many workers dating the gestation period from the time of estrus or copulation rather than the date of conception. Since the cow does not ovulate until the day following estrus and since conception cannot occur until after ovulation, for this study the length of gestation in the cow was measured from the day of ovulation. Because of the absence of reliable information on the time and sequence of events in bovine placentation, and the conflicts in interpretation of observed processes, an intensive study of the processes of bovine placentation was undertaken.

MATERIALS AND METHODS

The reproductive tracts studied were obtained from 23 cows

with known reproductive records culled from the Kansas State College dairy herd. The animals used were from the four common dairy breeds and all were determined to be clinically normal by rectal palpation before slaughter (Table 1). Most were slaughtered in order to obtain timed stages of embryogeny. Ages of the animals ranged from 2 to 12 years.

The cows were slaughtered at a local abattoir. The reproductive tracts were removed as soon as possible after slaughter, normally within 15 minutes. During the early stages of the study, tracts were placed in plastic bags and packed in chipped ice for removal to the laboratory where they were weighed, measured and dissected. Colored photographs were taken, after which the tissues were fixed by immersion in 10 per cent formalin (Table 1). Later in the study, after adequate photographs had been obtained, fixation was accomplished at the abattoir by perfusion of the tract via the uterine arteries with 10 per cent formalin immediately upon excision (Table 1). The latter technic gave much better histological fixation.

Whole caruncles and entire placentomes were removed routinely from each uterine horn from the area around the embryo or fetus. Complete sections through the entire uterine horn about 3 cm from the utero-tubal junction were removed from selected tracts after fixation. Tissue blocks were dehydrated in a series of graded alcohols, embedded in paraffin using the isopropyl alcohol technic and sectioned routinely at 10 microns.

Table 1. The source, history and fixation of the uteri studied.

Cow	Breed	Yrs:Mo	Reproductive status		Side of ovulation	Number of preg. or previous births	Times bred before slaughter	Method of fixation with 10% formalin
12	Ayrs.	2-7	Preg.	25	Right	1	1	imm.
83	Jers.	2-11	Preg.	26	Right	1	2	perf.aba.imm.
4	Jers.	5-7	Preg.	31	Right	3	3	perf.lab.imm.
20*	Hols.	3-9	Preg.	31*	Left	2	3	inj.aba.imm.
50	Jers.	12-7	Preg.	35	Left	9	5	imm.
73	Ayrs.	2-2	Preg.	38	Right	0	4	perf.aba.imm.
1	Guer.	8-3	Preg.	41	Right	6	4	perf.lab.imm.
75	Hols.	3-7	Preg.	43	Left	1	1	perf.aba.imm.
42	Jers.	2-1	Preg.	54	Left	0	6	imm.
60	Jers.	3-10	Preg.	63	Left	2	3	imm.
7	Hols.	2-10	Preg.	69	Right	1	1	inj.perf.lab.
10	Hols.	5-7	Preg.	72	Left	4	7	imm.
24	Hols.	3-0	Preg.	83	Right	1	4	imm.
2	Ayrs.	6-9	Preg.	90	Right	4	2	perf.lab.imm.
9	Jers.	2-0	Preg.	91	Right	0	1	imm.
13	Ayrs.	2-10	Preg.	93	Right	1	2	imm.
23	Jers.	3-2	Preg.	116	Right	1	3	imm.
6	Guer.	4-3	Preg.	148	Left	2	1	imm.
53	Hols.	10-9	Preg.	240	Left	6	1	imm.
51	Guer.	12-2	N B	0	Right	7	0	imm.
3	Ayrs.	7-4	N S	6	Left	3	8	perf.lab.imm.
5	Guer.	5-0	N B	15	Right	3	0	perf.lab.imm.
8	Jers.	3-8	N S	21	Right	1	2	imm.

N B = not bred; N S = not settled; imm. = immersed, 5 hrs. after slaughter; perf. = perfused through the uterine arteries; inj. = injected into the uterine cavity; aba. = at abattoir immediately upon excission; lab. = after returning to the laboratory.
* - twin embryos.

Selected tissues out of each horn were post-chromated with 2.5 per cent potassium dichromate for 24 hours at 48°C and then washed in running tap water overnight before being dehydrated and embedded in paraffin. Each chromated tissue had at least one formalin fixed tissue for histological comparison.

Sections were stained with a modified Harris' hematoxylin and eosin bluish as the counterstain at first. Later, a combination of acid fuchsin and orange G was used as the counterstain.

Dissections of the uteri and their contents were carried out under water. Placentomes were cut at the peduncle and allowed to remain attached to the chorio-allantois for complete fixation when fixed at the laboratory. Membranes of the embryos fixed immediately at the abattoir were anchored to the uterine wall around the placentome by sewing them together to keep the membranes in their natural position during the dissection, dehydration and embedding.

Since ovulation occurs on the average between 10 and 14 hours after the end of estrus, the arbitrary time of 12 hours after the end of estrus was chosen as the time of ovulation for all of the animals in this study. This made the morning of the day following estrus (the insemination date) the time of ovulation or day zero in the timing process.

Gross measurements were taken with vernier calipers. Microscopic measurements were made with the aid of a calibrated

ocular micrometer. The photographs in Plates II, III, IV were taken of the natural unfixed tissues immediately upon returning to the laboratory. Plates I, V, VI, VII, VIII, IX were composed from photographs taken after fixation of the tissues with 10 per cent formalin. The photomicrographs were taken using sectioned and stained tissues.

OBSERVATIONS

The Endometrium

The bovine endometrium contains four distinguishable layers (Plate I)*, (1) the uterine epithelium, (2) the stratum compactum, (3) the stratum spongiosum and (4) the stratum basalis. Uterine glands are found in the endometrium arising as many branched, coiled tubules in the stratum basalis. They pass as highly coiled tubules through the stratum spongiosum where the branches unite and then pass through the stratum compactum as a relatively straight tubule opening directly into the uterine lumen. The uterine epithelium is continuous with the uterine gland epithelium (Plate I).

Macroscopically, the endometrium presented a soft, generally wrinkled surface. During non-pregnancy the caruncles appeared as large bumps (Plate II, Fig. 1) or merely as small circular excrescences much like small volcanic craters (Plate II, Fig. 2). The size and appearance of the caruncles depended upon the age

* All plates are in the Appendix.

of the cow and the stage of the estrous cycle when slaughtered. Older polyparous cows tended to have larger caruncles than heifers and those animals just approaching or leaving estrus tended to have larger caruncles than those in mid-cycle.

Pregnant uteri up to the 26th day of gestation presented no observable change from that of the non-pregnant uteri of the same time post ovulation. However, less than a week later (31 day stage) a slight swelling of the gravid horn became noticeable (Plate III, Fig.1). The endometrium appeared somewhat edematous and those caruncles nearest the embryo showed a slight increase in diameter and a rugate surface. As pregnancy progressed, more and more caruncles in the gravid horn and finally in the non-gravid horn were observed to be stimulated. Development was gradual with the largest caruncles always nearest the embryo or fetus and the smallest toward the distal ends of the chorionic vesicle. The caruncles expanded into the lumen as larger and larger masses (Plate IV, Figs. 2, 3, 4). By the end of the third month, most of the caruncles had been stimulated in both the gravid and non-gravid horns (Plate III, Fig. 2).

Macroscopic examination of the caruncles after removal of the cotyledons revealed several small cryptal openings spread over the surface (Plate IV). At 240 days gestation the appearance of the placentomes was similar to that noted for the earlier stages. The only apparent change had been in size.

Since the presence or absence of the uterine epithelium could not be determined and internal changes could not be followed

by macroscopic observation, histological preparations were studied representing tissues from each uterus.

The Endometrium During Non-Pregnancy. The endometrium when considered as a whole changed little during the estrous cycle except for a noticeable edema during estrus. Microscopically, however, structural changes in the various layers were observed.

The Uterine Epithelium. The epithelium covering the endometrium showed some of the most extensive changes observed during the estrous cycle. Although present in all stages studied, the height of the epithelium fluctuated from 7 to 28 microns over the caruncles and from 21 to 35 microns over the intercaruncular areas (Table 2).

The epithelium on the day of ovulation (one day post estrus) was intact over the caruncular and intercaruncular areas. The cells were tall columnar and appeared pseudo-stratified. With proper adjustment of the light source, cell membranes were observable. The nuclei were dark staining and compressed in about three-fourths of the cells (Plate V, Fig. 1). The remaining one-fourth were oval to spherical, coarsely granular and pale staining, scattered throughout the epithelium in small groups. The nuclei over the caruncles appeared slanted to horizontal as if the cells were under tension from being stretched. The intercaruncular epithelium was seemingly unaffected since its nuclei were vertical and the cell height was three times that of the caruncular epithelium (Table 2).

Table 2. Observations of the uterine epithelium after fixation with 10% formalin and staining with acid hematoxylin.

Cow	Reproductive status		Caruncular Epithelium: Appearance of nuclei	Caruncular Epithelium: Thickness in microns	Intercaruncular Epithelium: Appearance of nuclei	Intercaruncular Epithelium: Thickness in microns
12	Preg.	25	---------	--	---------	--
83	Preg.	26	spher.gran.pl.	28	spher.gran.pl.	31
4	Preg.	31	spher.gran.pl.	28	spher.gran.pl.	21
20	Preg.	31	spher.gran.pl.	21	spher.gran.pl.	21
50	Preg.	35	---------	nm	spher.gran.pl.	21
73	Preg.	38	spher.gran.pl.	7	spher.gran.pl.	21
1	Preg.	41	spher.gran.pl.	21	spher.gran.pl.	21
75	Preg.	43	spher.gran.pl.	7	spher.gran.pl.	14
42	Preg.	54	spher.gran.pl.	7	spher.gran.pl.	nm
80	Preg.	63	spher.gran.pl.	7	spher.gran.pl.	nm
7	Preg.	69	spher.gran.pl.	7	---------	--
10	Preg.	72	spher.gran.pl.	7	---------	--
24	Preg.	85	spher.gran.pl.	7	---------	--
2	Preg.	90	spher.gran.pl.	7	spher.gran.pl.	21
9	Preg.	91	spher.gran.pl.	7	---------	--
13	Preg.	93	spher.gran.pl.	7	spher.gran.pl.	21
23	Preg.	116	spher.gran.pl.	7	spher.gran.pl.	21
6	Preg.	148	spher.gran.pl.	7	spher.gran.pl.	21
51	N B	0	com.ovl.opa.	7	spher.gran.pl.	21
3	N S	6	spher.gran.pl.	28	spher.gran.pl.	35
5	N B	15	spher.gran.pl.	21	spher.gran.pl.	21
8	N S	21	spher.gran.pl.	21	spher.gran.pl.	21

N B = not bred; N S = not settled; nm = not measured; spher. = spherical; pl. = pale staining; opa. = opaque to dark staining; ovl. = oval; com. = compact.

Six days later the epithelium was still intact and continuous over the caruncles and intercaruncular areas. Nuclei throughout were spherical, granular and pale staining (Plate V, Fig. 2). The cells were tall columnar with the nuclei located basally to centrally which gave the characteristic psuedo-stratified appearance. Cellular height was at its cyclic maximum, the intercaruncular epithelium being about 25 per cent taller than that over the caruncles (Table 2).

In the samples from the 15 day post ovulation uterus, the nuclei of the epithelium were oval to spherical, granular and pale staining in the intercaruncular areas but more compressed and slightly darker staining over the caruncles. No difference in cell height was noted between the two areas. Cell membranes were distinct. The layer still was tall columnar and psuedo-stratified (Plate V, Fig. 3).

At 21 days the epithelial nuclei were mostly oval, coarsely granular and pale staining. Cell membranes were distinguishable. The tall columnar cells retained the psuedo-stratified appearance over caruncular and intercaruncular areas alike.

The Stratum Compactum. Histologically the stratum compactum appeared as a distinct, eosinophilic layer just under the uterine epithelium (Plate I; Plate V, Figs. 1, 2, 3). It was continuous throughout the endometrium, even through the caruncles. The compactum was never observed as an edematous layer although underlying layers were greatly affected. Thickness of the stratum

compactum did not change appreciably throughout the cycle. During pregnancy, the caruncular stratum compactum became the base of the placentomes. The intercaruncular compactum remained unchanged except for being stretched.

The Stratum Spongiosum. Underneath the stratum compactum, the stratum spongiosum presented a distinctly different appearance (Plate I). The spongiosum contained fewer nuclei than the compactum and was distinctly less eosinophilic. Differentiation of the spongiosum from the underlying stratum basalis was indefinite. Edema was observed in the spongiosum but only when also present in the basalis. In the caruncles, a distinctly different tissue, the discus expansus*, was prominent between the stratum compactum and the stratum spongiosum.

The Stratum Basalis. The most basal part of the endometrium was the stratum basalis. Histologically, the layer appeared similar to the spongiosum and was inseparable from it. The basalis could be determined only by definition as "that portion of the endometrium in which the uterine glands terminate." It extended under the caruncles and intercaruncular areas as a continuous layer.

The Discus Expansus. Between the stratum compactum and the stratum basalis of the caruncle was an intensely staining area, the discus expansus (Plate V, Fig. 4), which caused the familiar caruncular bulge. The discus expansus was composed of

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* See Appendix.

many densely packed nuclei surrounded by very little but intensely staining cytoplasmic material. Interspersed throughout were capillary buds around which the nuclei of the discus were oriented (Plate VI, Figs. 1, 4). Larger blood vessels had larger areas of nuclei oriented around them. Such oriented nuclei were oval to compressed oval and stained more intensely than other endometrial nuclei. At stages during which the capillaries extended, the nuclei expanded, became lighter staining and migrated with the growing capillaries toward the lumen of the uterus. With this increase in the size of the nuclei, "flowing" streams of cells moving into the stratum compactum became apparent (Plate VI, Fig. 1).

The Endometrium During Pregnancy. Microscopically, many changes in some layers of the endometrium were seen during pregnancy that did not occur in the non-pregnant uterus.

The Uterine Epithelium. The uterine epithelium was present over the caruncles in all cases except one (Table 2). Where tissues were obtained for study of the intercaruncular epithelium, it was also observed to be present except in one case. The exception in both cases was from the same uterus and was directly attributable to improper handling and delayed fixation.

The height of the epithelium increased from 21 to 28 microns during the 20th to 30th days of pregnancy. By the 38th day, the epithelial height was reduced to 7 microns at the points of attachment (Plate VI, Fig. 3) (Table 2). Erosion of the

uterine epithelium by the serosal ectoderm was never observed. With the increase in size of the caruncle and accompanying stretching, the covering epithelium changed from pseudo-stratified tall columnar to simple cuboidal. The nuclei remained spherical, granular and pale-staining throughout the period. Once a placentome was established, the thickness of the epithelium lining the caruncular crypts did not vary in thickness. Where adequate and proper fixation was obtained, cell membranes were always present. Syncytia appeared only in those cases where fixation was delayed and/or inadequate.

The Stratum Compactum. The stratum compactum changed very little throughout pregnancy. It was noted as the most stable tissue of the edometrium. Changes observed were merely resultant of the stretching of the uterus to accommodate the growing embryo and its membranes. This actually made recognition of the layer easier since it retained its compactness and eosinophilia. With formation of the placentome, the caruncular stratum compactum became its base.

The Stratum Spongiosum and Stratum Basalis. The stratum spongiosum and stratum basalis were edematous at the 25 and 26 day stages. The stretching of the endometrium during the next six days resulted in reduction in the overall thickness of the strata. With the edema and stretching of the strata, what little differentiation there had been between the spongiosum and basalis was lost.

By 38 days gestation the strata were stretched until they were reduced to a very thin layer under the discuss expansus (Plate VI, Fig. 3).

The Discus Expansus. The discus expansus was the area of cellular activity and change during pregnancy. At 25 and 26 days the cells were very similar to those in corresponding caruncles of non-pregnant uteri. Later as embryonic stimulation increased, the vascular bed in the base of the discus expanded and pushed deeper into the discus and on into the stratum compactum. This brought about the migration of cells from the discus through the stratum compactum. These cells formed projections, the septa of the beginning placentomes, upon the caruncular surface by the 31st day (Plate VI, Fig.2; Plate IV, Fig. 1). Between the septa were the maternal or caruncular crypts.

As the placentomes grew, the cells of the discus expansus were incorporated into their septa. This resulted in the discus becoming progressively smaller as the placentome increased in size (Plate VII). By the 72nd day, the discus expansus was no longer observed as a definite structure under those placentomes of greatest development.

The Placenta

The development of the bovine placenta was gradual and continuous over a period of several weeks. Observations were

grouped into three periods; (1) before attachment, (2) during attachment and (3) later development and growth.

The Period Before Attachment. Two pregnant uteri were obtained that had no evidence of attachment. The uterine epithelium was present with varying heights. The serosal ectoderm of the embryo was in opposition to the uterine epithelium throughout the extent of the serosal sac (Plate VIII, Fig. 1). Fetal giant cells were noted throughout the serosal ectoderm but were mostly concentrated over the caruncles. Giant cells of maternal origin were also observed (Plate VIII, Fig. 1). Many fetal giant cells were mononucleate. Maternal giant cells were most often di- or polynucleate and did not stain as intensely as those of fetal origin.

The allantois of the 25 day embryo was well developed and expanded, but had not yet started to fuse to the serosa. Although the allantois was vascularized to a slight extent, no allantoic blood vessels had yet become embedded in the serosal mesoderm.

The Period During Attachment. The period during attachment was found to extend over a considerable length of time; from the appearance of the first maternal crypts and fetal villi until no new placentomes were formed. The observed length of time involved was from the 31st day until the 91st day of gestation. This period was sub-divided into the period of initial attachment and the period of later attachment.

The Period of Initial Attachment. This was defined as the time from the first appearance of fetal villi until attachment was firm enough to resist separation. The first observed villi appeared on the serosa immediately surrounding the embryo at 31 days (Plate VIII, Fig. 2). This coincided with the extent of fusion of the allantois to the serosa and hence the extent of the chorio-allantois. The villi formed first in rows perpendicular to the long axis of the chorionic vesicle and fit into corresponding crypts in the caruncle (Plate IV, Fig. 1). Villi formed only in the proximity of allantoic blood vessels.

By 35 days the caruncular septa had extended farther into the lumen making the crypts deeper. The edges of the septa became thicker so that in section a septum appeared club shaped with the small end attached to the stratum compactum. The villi responded by becoming longer and developing club shaped endings of their own. The expansion of the villi and the thickening of the cryptal lips effected an interlocking of the maternal and fetal tissues. The serosal ectoderm was in direct apposition (contact but not fusion) with the uterine epithelium.

By 38 days gestation the gravid horn had increased in diameter due to the increase in size of the chorionic vesicle, stretching the uterine wall into a thin layer (Plate VI, Fig. 3). This uni-directional stretching of the endometrium resulted in the expansion of the caruncle to a long oval structure rather than the round crater of non-pregnancy. The long axis of the stretched caruncle was parallel to the circular muscles (that

is, perpendicular to the long axis of the uterine lumen).

The discus expansus was still thick enough that even with the stratum spongiosum drastically reduced under it, it bulged towards the uterine lumen (Plate VI, Fig. 3). More and more nuclei in the discus appeared to expand and then migrate through the stratum compactum into the caruncular septa. This movement caused the formation of streams of similarly oriented nuclei "flowing" toward the septa along with the advancing maternal blood vessels. (Plate VI, Fig. 4).

The septa had expanded farther away from the stratum compactum than at 35 days and had branched forming smaller crypts within the walls of the original ones. The villi had branched and expanded accordingly. This branching of the villi brought about attachment firm enough to withstand separation of the tissues even after release of the pressure from the allantoic fluid. The period of initial attachment had ended. The time elapsed in the establishment of a placentome was about seven days.

The Period of Later Attachment. This was the time from the end of initial attachment to cessation of development of new placentomes. The development of additional places of attachment continued at a gradual but consistant rate. The number of discernable placentomes tripled from 31 to 41 days (Table 3). The newly formed placentomes were most distant from the embryo and coincided with the limits of allantoic blood vessel migration.

Table 3. The number and size of caruncles and placentomes in the gravid horn.

Cow	Days pregnant	Caruncles		Placentomes			
		Number[T]	Size in mm[#]	Number	Size in millimeters: Smallest	Largest	Average
12	25	56	3 x 4	none			
83	26	u.c.		none			
4	31	45	3 x 4	14	3 x 4	4 x 11	4 x 5
20*	31*	46	3 x 4	18*	2 x 2	6 x 9	4 x 7
50	35	44	3 x 4	13	3 x 5	5 x 6	4 x 5
73	38	u.c.					
1	41	50	2 x 7	40	2 x 5	3 x 11	4 x 8
75	43	55	##	55	3 x 4	6 x 10	5 x 7
42	54	u.c.		46	3 x 4	15 x 20	
60	63	u.c.		27	4 x 5	15 x 20	12 x 15
7	69	62	2 x 3	59	2 x 4	12 x 22	8 x 12
10	72	45	##	45	2 x 3	14 x 22	6 x 14
24	83	59	##	59	3 x 4	25 x 34	17 x 25
2	90	44	##	44	5 x 8	36 x 52	17 x 32
9	91	48	2 x 3	46	2 x 3	24 x 42	23 x 31
13	93	64	##	64	3 x 5	19 x 42	19 x 27
23	116	53	##	53	12 x 15	34 x 47	23 x 32
6	148	u.c.	##	55	12 x 15	40 x 62	32 x 45
53	240	58	##	58			45 x 90

T -- total number of caruncles.
-- diameter of the discoid surface of caruncles not involved in placentomes.
u.c. -- uncountable after fixation.
* -- twin embryos.
-- sizes listed under placentomes.

These placentomes were in a stage of initial attachment similar to that observed at 31 days near the embryo. Both villi and septa were simple and unbranched. They were held together only by the pressure of the expanding chorionic vesicle. The first observed development of attachments in the non-gravid horn was at 43 days (Table 4). Once the development had started in the non-gravid horn it continued as consistently as it had in the gravid horn, stopping only when the limit of the chorio-allantois had been reached.

The Period of Later Development and Growth. This was defined as that period of time after the end of initial attachment until parturition. As a consequence, those placentomes nearest the fetus necessarily had a longer period of development and growth than those that were formed last near the ends of the chorionic vesicle. By the 41st day of gestation, it was noted that the caruncular septa had continued to elongate and branch. This produced new crypts and more space in the original crypts. With the added space, the villi (Plate VIII, Figs. 3, 4) elongated and branched accordingly. The tips of the villi appeared to stay in contact with the uterine epithelium next to the stratum compactum where they first touched. The growth and elongation came about by proliferation of new ectodermal cells from the arcade zone of the cotyledons (Plate IX, Figs. 2, 3). The cotyledon at 116 days gestation (Plate IX, Fig. 1) presented an immense increase of fetal tissue beyond that which was present

Table 4. The number and size of caruncles and placentomes in the non-gravid horn.

Cow	Days pregnant	Caruncles		Placentomes			
		Number[T]	Size in mm[#]	Number	Size in millimeters Smallest	Largest	Average
12	25	48	5 x 6	none			
83	26	u.c.					
4	31	48	3 x 3	none			
20[*]	31	46	4 x 5	none			
50	35	48	3 x 3	none			
73	38	u.c.					
1	41	52	4 x 4				
75	43	48	3 x 3	6	3 x 4	5 x 7	3 x 5
42	54	u.c.		6			4 x 5
60	63	u.c.		16	3 x 4	6 x 15	5 x 12
7	69	u.c.		82	2 x 3	8 x 20	6 x 10
10	72	50	3 x 6	7	3 x 4	3 x 12	3 x 9
24	83	50	2 x 3	42	2 x 3	10 x 15	10 x 10
2	90	45		8			
9	91	52	3 x 4	44	4 x 5	15 x 23	13 x 18
13	93	45	4 x 4	18	2 x 4	10 x 20	7 x 13
23	116	32	##	32	7 x 10	19 x 35	16 x 25
6	148	u.c.					
53	240	u.c.		29			30 x 52

T -- total number of caruncles.
-- diameter of the discoid surface of caruncles not involved in placentomes.
u.c. -- uncountable after fixation.
* -- twin embryos.
-- sizes listed under placentomes.

at 31 days (Plate VIII, Fig. 2), accounting for a large portion of the increase in size of the placentomes.

The observable changes during the period of later development and growth were largely increases in size (Tables 3, 4) (Plate IV). Histological changes were slight. The septa were slightly thinner but the uterine epithelium lining the crypts remained unchanged (Table 2). The villi branched more and more giving greater surface contact with the cryptal lining (Plate VIII, Fig. 4; Plate IX, Fig. 2). Large mesodermal cores developed within the main villi thereby increasing the amount of fetal tissue present, and allowing larger allantoic blood vessels to penetrate into the placentome.

DISCUSSION

This investigation followed the development of the bovine placenta from the time of initial attachment until 240 days gestation. Changes in the structure and size of the placentomes occurred rapidly during the period of attachment but became progressively slower as gestation advanced. The material used was obtained from dairy cows that were slaughtered for the express purpose of collecting timed stages of embryogeny with the ensuing placental story and thus the material can be considered normal.

The exclusive use of dairy breeds for this study was not unique since Weeth and Herman (1952) also limited their investigations in this manner. However, the number of gravid uteri in

the present study was slightly over twice as many as previously reported for dairy cattle and about the same as reported in the beef breeds (Melton, et al., 1951; Foley and Reece, 1953) except for Björkman (1954) who reported on 91 abattoir specimens of unspecified breeds.

The methods of obtaining tissue samples for histological study were as varied as the number of workers. All too frequently the methods as reported were far removed from those generally accepted as giving satisfactory histological results. Part of this difficulty was due to the size of the cow as an experimental animal and the consequential elapse of time between slaughter and actual removal of the uterus. The real problem, however, seemed to evolve into the treatment of the uterus after removal. It was indeed striking to note that when the authors reported the method of opening the uterus and removing tissue blocks before fixation, they have also reported the loss or partial loss of some specific part or layer (i.e., the uterine epithelium) (Melton, et al., 1951; Weeth and Herman, 1952; Foley and Reece, 1953). Björkman (1954) noted the loss of tissue by this method and very wisely fixed his material thoroughly before attempting dissection. The observations of this study have reinforced the findings of Björkman (1954) in this respect and in addition have indicated that even with perfect fixation, the tissue may be damaged during the dissection, dehydration and embedding, particularly in the cases of early attachment. To further avoid

this damage of tissue and particularly to prevent the parts of the early placentomes from separating, the technic of sewing the fetal membranes to the uterine wall (Wimsatt, 1950) was tried. This was done after fixation, usually before dissection and was found to give excellent results. The greatest difficulty was in locating a placentome and then keeping the stitching at least 15 mm. distant to prevent the needle or thread from disrupting the membranes. Once this technic was developed, no further trouble with the separation of the tissues was encountered.

Quite by accident, one case in the study died while on pasture instead of by slaughter. Consequently the maternal blood was not drained from the uterus as in normal slaughter. Upon delayed receipt of the uterus, it was immediately perfused via the uterine arteries and fixative was injected into the allantoic sac. Study of the prepared sections later showed that this tissue was in better condition than any before although fixation had not occurred until 14 hours after death of the animal. It is definitely not recommended that fixation be delayed even for a few minutes; rather it is suggested that if some technic were to be devised whereby the maternal blood and blood pressure could be retained in the uterus until after fixation, much more satisfactory results would be obtained with less shrinkage of the caruncular tissue.

The endometrial changes followed regular cyclic patterns. Observation of the non-pregnant endometrium during this study

agreed closely with previous studies (Cole, 1930; Asdell, et al., 1949; Weeth and Herman, 1952; Foley and Reece, 1953). The increase in thickness was resultant from edema of the stratum spongiosum and the increased size of the vascular bed. The endometrium during pregnancy underwent changes during the first 25 to 26 days similar to those observed during the estrous cycle. By the 31st day, however, the presence of the growing embryo and its expanding chorionic vesicle was exerting pressure on the endometrium resulting in the compression and then stretching of the endometrial strata. The continued expansion of the chorionic vesicle resulted in additional thinning of the endometrial strata as shown in Plate VI, Fig. 3 at 38 days gestation. The stratum spongiosum and stratum basalis were compressed together until it was impossible to determine any difference between the two layers. The uterine glands were lying generally parallel to strata instead of relatively perpendicular as during the estrous cycle.

The stratum compactum in the caruncle has been described as being in two layers (Weeth and Herman, 1952; Björkman, 1954) the deeper stratum compactum described by Björkman (1954) was designated as the "discus expansus" in this study (see Appendix). It was unclear as to which part was meant by Weeth and Herman (1952) in their description of the changes in the caruncular stratum compactum. The description as given of certain nuclei being large, ovoid and rather vesicular agreed closely with

present observations of the nuclei of the discus expansus rather than the stratum compactum. They did, however, note that the stratum compactum remained dense during gestation which was borne out by this study.

The discus expansus underwent progressive cyclic changes of expansion and contraction during the estrous cycle. The increase in the size and number of blood vessels in the subcaruncular vascular bed resulted in stimulation of the nuclei of the discus to expand thereby contributing to the overall increase in size of the caruncle during estrus. This increase in the size of the discal nuclei surrounding the advancing blood vessels brought about the formation of visible streaks or "streams" of cellular activity as shown in Plate V, Fig.4 and Plate VI, Figs. 1,4. The streams of nuclei moved into and then withdrew from the overlying stratum compactum during the estrous cycle. During pregnancy the streams continued through the compactum in response to stimulation from the embryo and the migrating nuclei formed the septa of the developing placentomes (see below).

The uterine epithelium has generally been designated as pseudo-stratified tall columnar (Cole, 1930; Hatch, 1941; Weber, Morgan and McNutt, 1948; Asdell, et al., 1949; Roark and Herman, 1950; Weeth and Herman, 1952) but some have reported it as simple columnar of varying heights (Foley and Reece, 1953). The observations of this study have agreed that the epithelium

is indeed tall columnar with a pseudo-stratified appearance. The nuclei of the epithelium underwent changes as observed by Cole (1930) and Roark and Herman (1950). The result of observation on pregnant uteri are considerably different than most of those views previously published. The uterine epithelium has been described as being lost or eroded during pregnancy (Hallman, 1924; Hallman, 1925; Melton, et al., 1951; Weeth and Herman, 1952; Foley and Reece, 1953; Moss, et al., 1956b). Björkman (1954) suggested instead that the epithelium remained intact and Hatch (1941) stated positively that the uterine epithelium did remain intact and could be demonstrated as such. The observation of adequately fixed tissues during this study indicated that Hatch (1941) was correct. The uterine epithelium was observed to be intact at all stages of pregnancy studied. Erosion or penetration of the uterine epithelium by the serosal ectoderm was never observed.

The sequence of events initiating the formation of a placentome has been described as starting with an embryonic stimulation (Melton, et al., 1951; Foley and Reece, 1953). These authors noted the formation of the first fetal villi on the chorio-allantois ventral to the embryo. Melton, et al. (1951) suggested that possibly the pressure of the chorionic vesicle and its contents was the stimulus for formation of the villi. Foley and Reece (1953) believed the villi formed in shallow pockets of the caruncle and then penetrated the caruncular tissue.

Contrary to previous authors, the initial changes in the formation of a placentome were determined to occur only in the presence of an embryonic blood vessel growing through the chorio-allantois over the caruncle. No caruncles were observed to be stimulated unless covered by chorio-allantois (the fused serosa and allantois) in which a blood vessel (or vessels) was present. Stimulation from the fetal blood in the overlying embryonic blood vessel (or vessels) resulted in the further outgrowth of the capillaries and streams of nuclei from the discus expansus, forming caruncular septa as shown in Plate IV, Fig. 1 and Plate VI, Fig. 2. The serosal ectoderm then responded to the increased nearness of the caruncular capillaries and proliferated, forming villi in the pre-formed caruncular crypts between the septa.

The sequence of events leading to the formation of a new placentome appeared to be as follows: (1) an embryonic (allantoic) blood vessel passed over a caruncle, (2) the caruncle was stimulated by the nearness of the fetal blood and responded by forming septa on the caruncular surface, (3) the serosal ectoderm of the chorio-allantois then responded to the increased nearness of the maternal blood and proliferated forming villi that filled the caruncular crypts between the septa. The caruncular epithelium remained intact, lining all crypts! The cryptal walls (septa) extended into the lumen away from the stratum compactum. The stratum compactum remained as the base

of the placentome but did not enter into its formation (Plate VI, Fig. 2; Plate VII).

The formation of the placenta progressed in an orderly manner once it had been started. New placentomes formed distally from the embryo until all available caruncles had been used. The extension of the placenta was a gradual and continuous process. The first fragile attachments were found on the ventral surface of the chorio-allantois at 31 day post ovulation and new attachments were observed in all specimens studied up to the 91st day of gestation. With the study of properly fixed material, the first attachments could certainly be found by 30 days and probably by 29 days gestation. As placentomes completed their initial attachment, they continued to grow and expand. This meant that those placentomes formed first, functioned for the longest time and became the largest in size (Plate III, Fig. 2). The number of placentomes developed seemed to be consistent with the number of available caruncles. Winters, et al. (1942) reported that the bovine implanted by the 11th to 12th day of gestation (erroneously calculated). This conclusion was based on their inability to flush the embryo from the uterus and, evidently, their preconceived idea that an embryo had to implant (embed) in the endometrium. The more accurate observations of Melton, et al. (1951) and Foley and Reece (1953) that the embryo attaches rather than implants was borne out strongly by the observations

of this study. A little of the variation in the time of attachment as reported by these authors could be accounted for by the differences in the aging methods used. Dating timed stages from the day of insemination or from the day of ovulation results in different times since the day of estrus and the day of ovulation have been proven to be different in most cows (Trimberger, 1948). With this variation in mind, the times of attachment as reported by Melton, et al. (1951) and Foley and Reece (1953) agreed with the findings of the present study.

Although the placenta of the bovine has been classically defined as syndesmo-chorial, some recent investigators have offered evidence that did not agree with the old interpretations (Hatch, 1941; Wimsatt, 1951; Björkman, 1954). The question of classification has dealt solely with the presence or absence of a tissue normally only one cell thick, the uterine epithelium. The presence of the epithelium has been noted in all studies of the non-pregnant uterus but not so consistently in the pregnant one. The evidence as observed during this study indicated that the epithelium was present at all stages of gestation and therefore, the placenta of the cow should be classified as epithelio-chorial.

SUMMARY

Gross and histological observations were made on timed stages of placentation with 4 non-pregnant and 19 pregnant uteri

included in the study.

1. A clarification of terminology established the parts of the bovine placenta as the cotyledon (fetal) and the caruncle (maternal) with the union termed the placentome.

2. The endometrium was determined to consist of four rather distinct layers (stratum basalis, stratum spongiosum, stratum compactum, uterine epithelium). Thirty to 80 specialized areas, the caruncles, occur in each uterine horn. The caruncle differs from the rest of the endometrium by having no uterine glands, the discus expansus between the stratum spongiosum and the stratum compactum, and a distinct vascular bed in the spongiosum under the discus expansus.

3. The stratum compactum of the caruncle remained dense throughout gestation. It did not take part in the formation of the placentomes but rather became the base of them in the caruncular areas.

4. The cells of the discus expansus became directly the caruncular portion of the placentome (septa), penetrated by blood vessels from the underlying capillary bed.

5. The uterine epithelium was never eroded or lost but remained intact throughout gestation.

6. The placenta of the cow was determined to be epitheliochorial in structure.

7. The first fragile attachments were observed at 31 days gestation and became firm enough to resist separation by the 38th day of gestation.

8. New placentomes were formed until about the 91st day of gestation. Growth and expansion of the placentomes was still in progress at the 240th day of gestation.

ACKNOWLEDGMENTS

Acknowledgment and sincere thanks are given to Dr. H. T. Cier for his instruction, supervision and guidance coupled with extreme patience during this study.

Appreciation is also extended to Dr. G. B. Marion of the Department of Dairy Husbandry for his helpful suggestions and criticisms throughout the study.

The author is also deeply indebted to and wishes to thank the Department of Zoology for the laboratory space and equipment, the Department of Dairy Husbandry for the animals used and Kansas State Agricultural Experiment Station Project 426 for the necessary funds made available.

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APPENDIX

The use of the term "discus expansus" in reference to a specific part of the bovine uterine anatomy was not found in the literature. Previous workers have referred to the enlarged and predominantly dense portion of the caruncle as an extremely dense connective tissue (Cole, 1930) or as a portion of the stratum compactum (Weeth and Herman, 1952; Björkman, 1954). A lighter staining area has also been noted between the dense area and uterine epithelium of the caruncles (Cole, 1930) which was called the clear superficial zone. Some have noted that the dense caruncular area seemingly had proliferative powers (Weeth and Herman, 1952) but most authors merely designated the area as "that area characteristic of the caruncle."

Early observations during this study indicated that the light staining area just under the caruncular epithelium was in fact a continuation of the stratum compactum as was interpreted by Weeth and Herman (1952) and Björkman (1954). The "denser stratum compactum" referred to by Weeth and Herman (1952) lacked the characteristics of the rest of the stratum compactum. Instead, the area had distinct characteristic of its own such as darkly staining nuclei packed closely together, very little but very condensed cytoplasm and a large number of capillaries with nuclei of the area oriented around them. Closer observations showed this area to be distinctly separate from the stratum compactum and situated between the compactum

and the spongiosum during non-pregnancy.

The term "discus" was chosen in view of the plate-like shape of this characteristic area of the caruncle. Although the discus was not always flat, this was its most common condition. The adjective "expansus" was chosen as the most appropriate word descriptive of the action of the discus during the processes involved in placentation.

Explanation of Plate Abbreviations

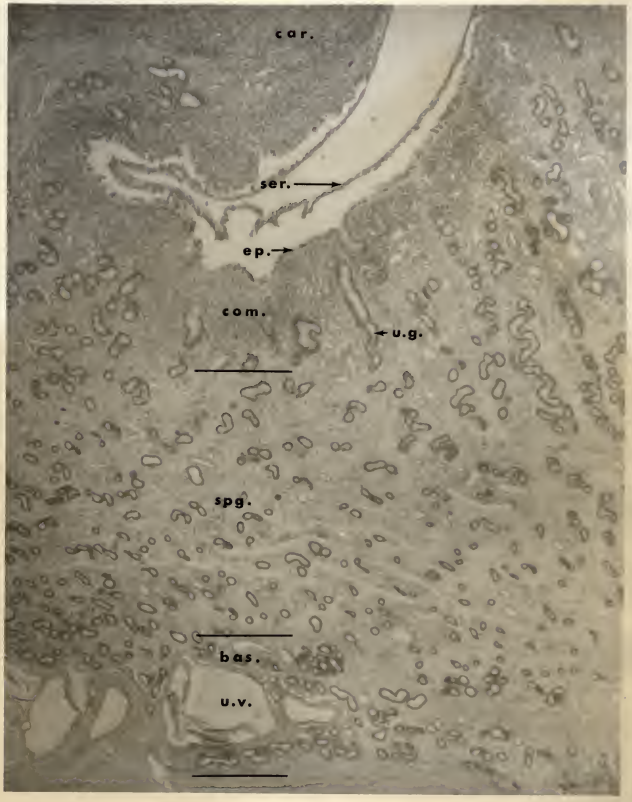
The photographs in Plates II, III, IV were taken of the natural tissue before fixation. Photographs in Plates I, V, VI, VII, VIII, IX were taken after fixation in formalin. Photomicrographs were taken of sectioned material stained with acid hematoxylin. The magnifications given are of the material as printed. The following abbreviations are used throughout:

al.v. --allantoic blood vessels.
arc. -- arcade zone of the placentome.
bas. -- stratum basalis.
car. -- caruncle.
ch. -- chorio-allantois.
com. -- stratum compactum
cot. -- cotyledon.
cr. -- caruncular crypt.
dis. -- discus expansus.
ect. -- serosal ectoderm.
ep. -- uterine epithelium.
mes. -- serosal mesoderm.
ser. -- serosa.
sep. -- septum.
spg. -- stratum spongiosum.
u.g. -- uterine gland.
u.v. -- uterine blood vessel.
vil. -- villus.

EXPLANATION OF PLATE I

Section through the endometrium at 38 days gestation, non-gravid horn. (50 X).

PLATE I



EXPLANATION OF PLATE II

Fig. 1. The opened uterus on the day of ovulation. Note the raised caruncles due to hypertrophied caruncular blood vessels and discus expansus. (0.40 X).

Fig. 2. The opened uterus at 6 days post ovulation. Note the reduced size of the caruncles. (0.48 X).

PLATE II

Fig. 1

Fig. 2

EXPLANATION OF PLATE III

Fig. 1. The opened uterus at 31 days gestation. Note the difference in size of the caruncles where attachment was in progress. (0.32 X).

Fig. 2. The opened uterus at 116 days gestation. Note the variations in the size of the caruncles indicative of the gradual process of placentome development. (0.29 X).

PLATE III

Fig. 1

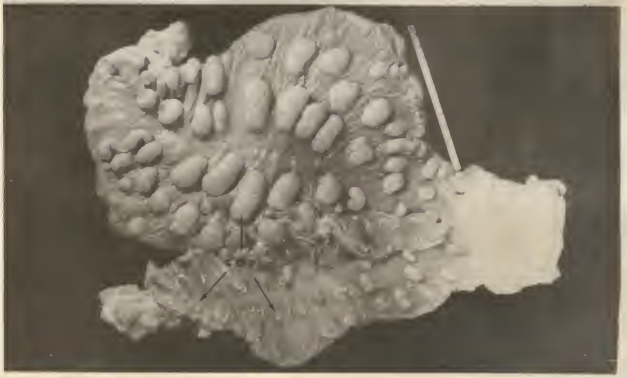


Fig. 2

EXPLANATION OF PLATE IV

Fig. 1. A caruncle at 31 days gestation. Note the rugosity of the caruncular surface.(5 X).

Fig. 2. Caruncles at 54 days gestation. Note the ridges and folds still evident. (2 X).

Fig. 3. Caruncles at 83 days gestation. Note the loss of folds and the increased size of the cryptal openings. (1.1 X).

Fig. 4. Caruncles at 116 days gestation. Note the overall increase in size. (1 X).

PLATE IV

Fig. 1

Fig. 2

Fig. 3

Fig. 4

EXPLANATION OF PLATE V

Fig. 1. The uterine epithelium over a caruncle at the day of ovulation. (1200 X).

Fig. 2. The uterine epithelium over a caruncle at 6 days post ovulation. (1200 X).

Fig. 3. The uterine epithelium at 15 days post ovulation. (1200 X).

Fig. 4. Section through a caruncle at 31 days gestation showing the edge of the discus expansus. (30 X).

PLATE V

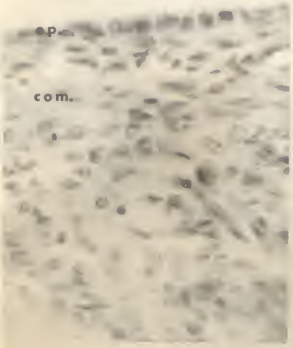


Fig. 1

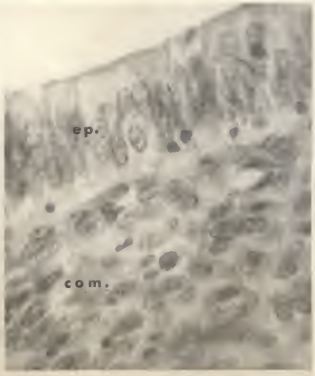


Fig. 2

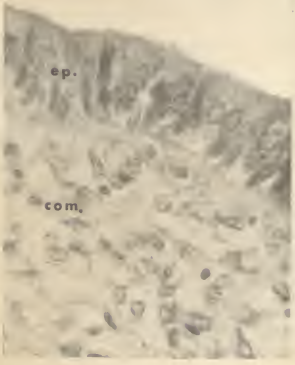


Fig. 3

Fig. 4

EXPLANATION OF PLATE VI

Fig. 1. Section through a caruncle at 26 days gestation showing the flowing of the cells of the discus expansus. (500 X).

Fig. 2. Section through a caruncle at 31 days gestation showing the maternal septa and crypts. Note lack of epitholium due to removal of the fetal membranes and excessive handling before fixation. (100 X).

Fig. 3. Section through an entire placentome at 38 days gestation. (28 X).

Fig. 4. Enlargement the square marked in Fig. 3 showing the streaming of cells from the discus expansus into the stratum compactum. (600 X).

PLATE VI

Fig. 1

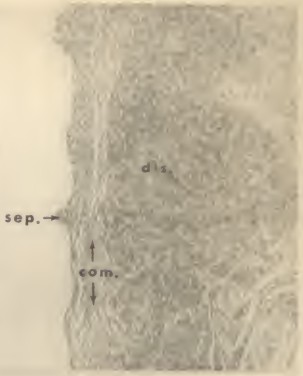


Fig. 2

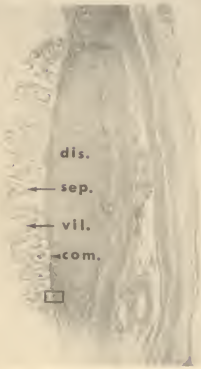


Fig. 3

Fig. 4

EXPLANATION OF PLATE VII

Fig. 1. Longitudinal section through an entire placentome at 38 days gestation. (35 X).

Fig. 2. Longitudinal section through an entire placentome at 43 days gestation. (28 X).

Fig. 3. Longitudinal section through an entire placentome at 69 days gestation. (28 X).

PLATE VII

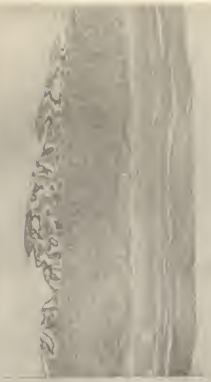


Fig. 1

Fig. 2

Fig. 3

EXPLANATION OF PLATE VIII

Fig. 1. Section showing the relationship of the serosa and endometrium before attachment, from the non-gravid horn of a 38 day gestation. (600 X).

Fig. 2. A portion of the chorionic vesicle at 31 days gestation to show the beginning cotyledons. (3 X).

Fig. 3. Section through a cotyledon at 41 days gestation showing the early branching of the villi. (100 X).

Fig. 4. Section through a cotyledon at 83 days gestation showing the increased branching of the villi. (50 X).

PLATE VIII

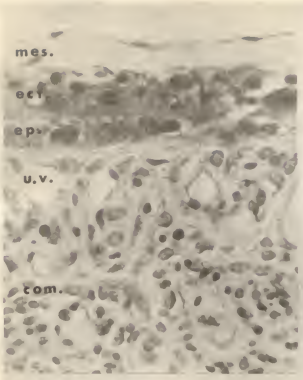


Fig. 1

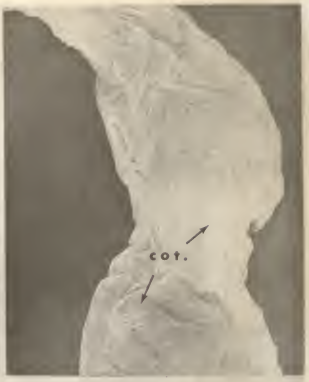


Fig. 2

Fig. 3

Fig. 4

EXPLANATION OF PLATE IX

Fig. 1. Macroscopic view of a cotyledon at 116 days gestation. (1.2 X).

Fig. 2. A portion of a longitudinal section through a placentome at 90 days gestation. (35 X).

Fig. 3. Increased magnification of the area marked in Fig. 2 to show the arcade zone and definite presence of the epithelium, even around the ends of the septa. (120 X).

PLATE IX

Fig. 1

Fig. 2

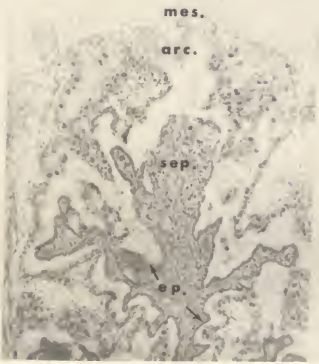


Fig. 3

PLACENTATION IN THE COW

by

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B. A., Park College, 1955

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1957

A study was made of the uteri from 19 pregnant and 4 non-pregnant dairy cows with known reproductive histories and timed stages of placentation. Observations were made on the gross appearance of the placenta. Details were observed using formalin fixed and acid hematoxylin stained histological sections of entire placentomes, caruncles and cotyledons.

The area of dark staining tightly packed nuclei and capillaries in the caruncles was observed to transform directly into the caruncular septa and was designated the discus expansus as a result of its general shape and behavior.

The first observance of fetal villi was at 31 days gestation (post ovulation). Attachment was firm enough to resist separation of the cotyledon from the caruncle by 38 days gestation. The development of new placentomes continued progessing distally from the embryo until about the 91st day of gestation. The placentomes were still increasing in size by the 240th day of gestation.

The placentome was formed as follows: (1) as the allantois fused with the serosa, allantoic blood vessels passed over caruncles in their growth through the chorio-allantois; (2) the caruncle responded to the proximity of the fetal blood with an outgrowth of caruncular septa formed from the capillaries and the nuclei of the discus expansus; and (3) the serosal ectoderm responded to the increased proximity of the maternal blood by proliferation of its cells resulting in formation of the fetal villi. The stratum compactum formed the base of the

placentome. The villi did not penetrate the caruncular tissue at any time. The uterine epithelium was found to be intact at all stages of gestation. The cells lining the maternal (caruncular) crypts were of maternal origin, i.e., the uterine epithelium, and hence, the placenta of the cow was determined to be epithelio-chorial.